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COMPARATIVE ANALYSIS OF DIFFERENT ELICITORS AND BINDERS AND DEVELOPMENT OF HERBAL FORMULATION CONTAINING CASSIA FISTULA L. FRUIT PULP AGAINST ALTERNARIA SOLANI

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ABSTRACT

In the present study various concentration (10% to 100%) of elicitors like neem, mustard and coconut oil cake and binders like cow dung, guar gum and gum acacia were used with the aim to study impact of concentration on anti fungal activity. 20gm elicitors and binders were dissolved in 100 ml of autoclaved water for 24 h. The mixture was then filtered and used for antifungal activity. Results suggested that antifungal activity increased with increasing concentration of elicitors and binders. Maximum activity was observed with 100% Concentration. Hence this concentration was used for the preparation of herbal formulation. 30 ratios of herbal formulations were prepared and assayed for antifungal activity against Alternaria solani by poison food technique. Among the formulations prepared optimum activity was observed for formulation number 8, 12, 18 and 22 i.e. 62.92%, 62.05%, 92.62%, 95.24% respectively against Alternaria solani. On the basis of results obtained, best herbal formulation will be used for in vivo experiments. The purpose of this work was to evaluate antifungal activity of herbal formulation against Alternaria solani, which cause early blight disease in potato crop.

KEYWORDS: Herbal formulation, Elicitors, Binders, Oil Cakes, Cow Dung.

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Original Article

INTRODUCTION

India is the largest manufacturer of medicinal plants and is called as botanical garden of the world (Seth & Sharma, 2004). In the last few years there has been an exponential development in the field of herbal drugs and these medicines are gaining popularity in all over the world because of their less side effects and natural origin . Medicinal plants are widely distributed in different region of India and compose important components of flora. Attention need to be given to assess the medicinal value of such plants to explore the potential drugs out of it. Many ancient medicines in use are obtained from medicinal plants, minerals and organic matter (Grover et al, 2002). In herbal preparations of Indian traditional health care systems the large number of medicinal plants are present which are used traditionally for over 1000 years named rasayana (Scartezzini & Sproni, 2000). Most practitioners formulate and dispense their own recipes in Indian systems of medicine (Seth & Sharma, 2004). Traditional natural products are effective sources of new agrochemicals for control of plant diseases (Kagale et al. 2004, Maya and Thippanna, 2013). Use of plant based products in agriculture is an environmentally safe and economically viable strategy for the control of diseases. Bio–agents and herbal product preparations do not leave any toxic residues and therefore can effectively replace synthetic fungicides.

Cassia fistula (Linn.) is a common plant known for its medicinal properties are a semi-wild in nature and belongs to family Fabaceae and Sub–family Caesalpinioideae. It is distributed in various regions including Asia, South Africa, China, West Indies and Brazil (Prashanth et al., 2006; Bhalerao et al., 2012). This plant has been extensively used in Ayurvedic system of medicine for various ailments, is commonly known as Amaltas and in English popularly called "Indian Laburnum". It is deciduous and mixed-monsoon forests throughout greater parts of India, ascending to 1300 m in outer Himalaya, is widely used in ancient medicinal system of India (Gupta, 2010). The plants are used in folk remedies for tumors of the glands, abdomen, liver, stomach, and throat cancer carcinomata, and impostumes of the uterus. Root is useful in heart diseases, fever, retained excretions and biliousness (Nadkarni, 2009).

Potato (*Solanum tuberosum* L.) is grown in about 140 countries and fourth important crop worldwide by volume of production; it is high yielding and having a high nutritive value (Malik and Tufail,1984). Potato is an cheap food and a source of low cost energy to our diet. This crop is excessively susceptible to early blight caused by *Alternaria solani*. Foliar symptoms of early blight disease first appear small, irregular to circular dark brown spots on the lower (older) leaves, too much defoliation may lead to death of the plant and high yield loss. The *Alternaria solani* can also attack potato tubers and symptoms are circular to irregular lesions that are slightly sunken and surrounded by a raised purple to dark brown border and produce a shallow, dry, corky rot (Folsom and Bonde, 1925, O'Brien and Rich, 1976, Wharton and Kirk, 2007).

In plant biology elicitors are extrinsic, or foreign, molecules often associated with diseases or synergistic organisms and plant pests. Molecules of elicitor can attach to specific receptor proteins located on plant cell membranes. The molecular pattern of elicitors are recognized by receptors and trigger intracellular defence signalling via the Octadecanoid pathway. The response results in the enhanced synthesis of metabolites which decrease damage and increase resistance to pest, disease or environmental stress (Bektas & Eulgem, 2015). Oil cake is one of the natural organic fertilizers with high nitrogen content, which is the residues of neem seeds, mustard, peanut seeds, sesame, coconut etc. after oil extraction process of the processing plant.

Neem (*Azadirachta indica*) is a monumental tree of Meliaceae family coming from the Indian subcontinent. Actually, distribution and importance of neem is increasing all over the World due to its important and beneficial properties, which reported by WHO/UNEP1989. Neem is considered to be one of the most valuable trees of the 21st century for its great potential in pest management, effective source of environmentally powerful natural pesticides, environmental protection and medicine. The waste by-product remaining after the oil extraction processes is neem cake. Neem is considered devoid of toxicity, as tested also by the old traditional use. Neemo cake has been successfully utilized as livestock feed for growing goats (Rao et al., 2003).

Binder is a material which holds or draws other materials together to form a cohesive whole mechanically, chemically, or as an adhesive. Generally materials labeled as binders in various proportions or uses can have their roles reversed with what they are binding. Guar gum, also known as guaran, is primarily the ground endosperm of guar beans. The guar seeds are dehusked, milled and screened to get the guar gum. It is produced as a free-flowing, off-white powder. The color of guar gum powder is whitish and yellowish having slight odor. Acacia gum has long been used in everyday applications and in traditional medicine. The material is used by Egyptians as glue and as a pain-reliever base. Arabic physicians with the gum treated a wide variety of ailments, resulting in its current name (Dobelis, 1986). Presently, it is used widely in the cooking industry to give body and texture to processed food products and in the pharmaceutical industry

as a demulcent. It is also used to stabilize emulsions. The bark fibers are used to make cordage (Duke, 1985).

Cattle rearing in India has been a tradition and intimately limited to agricultural economy. Different products used widely in number of Ayurvedic formulations are obtained from cow milk, ghee, curd, urine, and dung. In Indian sub-continental farming cow dung is traditionally used as organic fertilizer for centuries. The addition of cow dung increases the mineral status of soil, also increases resistance of plant against pests and diseases; increase plant growth and other beneficial activities such as sulphur oxidation and phosphorous solubilization. The Composition of cow dung is around 80% water and supports a matrix of undigested plant material that is rich in nutrients, micro-organisms, and their byproducts (Naskar & Ray, 2003).

There are several traditional agricultural practices followed by farmers to control plant diseases. Probably the oldest document was Kautilya's Arthasastra, which reported the use of organic materials to control the crop disorders. Formulation is a cheap, environmentally safe fungicide made by combining plant extracts and organic materials to control plant diseases. Many of such techniques of traditional agriculture that require validation, such as use of organic materials (cow dung, oil cakes etc.) for control of plant diseases (Nene, 2003). The present study has been done to develop herbal formulation from *Cassia fistula* L. fruit pulp extract in combination with neem oil cake and cow dung for control of early blight of potato caused by *Alternaria solani.*

MATERIALS AND METHODS

Preparation of Extracts

- 100% alcoholic Crude extract of *Cassia fistula* L. was prepared by according to the modified cold extraction method suggested by Shadomy and Ingraff, (1974). 20 gm dried and powdered plant material was suspended in 100 ml of solvent (100% alcohol) for 24-48 hrs. Whatman filter paper no.1was used for filtration of suspension then vacuum dried with the help of rotary vacuum evaporator.
- Partially purified chloroform extract was prepared by according to the hot extraction method suggested by Harborne, (1984). 40 gm dry plant powder was kept in Soxhlet extraction unit and extracted with 280 ml solvent.
- 20gm elicitors like neem, mustard and coconut oil cake and binders like cow dung, guar gum and gum acacia were dissolved in 100 ml of autoclaved water for 24 h. The mixture was then filtered and used for antifungal activity.

Antifungal Activity of Elicitors and Binders Individually

10% to 100% solution of elicitors like neem, mustard and coconut oil cake and binders like cow dung, guar gum and gum acacia were prepared and assayed for antifungal activity against *Alternaria solani* by poison food technique. Various concentrations (10% to 100%) of elicitors and binders were used with the aim to study impact of concentration on antifungal activity.

Preparation of Herbal Formulations

Herbal formulations were prepared by using plant extracts, elicitor and binder in different ratio. 100% of neem oil cake and 100% of cow dung were mix with 100% alcoholic and partially purified extract. All ingredients of herbal formulation were used in following ratio:

- Crude extract : elicitor : binder
- Partially purified extract : elicitor : binder

Antifungal Activity of Herbal Formulations

Antifungal activity of 30 formulation ratios was done by poison food technique against *Alternaria solani* (Groover, & Moore, 1962). 9 ml of molten PDA medium was poured into test tubes and then autoclaved. The molten sterilized medium along with 1 ml of formulation (extract: neem oil cake: cow dung) was placed into Petri plates and in the control set no formulation was used. After the solidification of the media, 6mm inoculum disc of 7 days old culture of the fungus was aseptically inoculated upside down in the centre of the Petriplate and incubated at 25±2ºC.

On the 7^{th} day of incubation average diameter of the fungal colonies was measured and percentage of mycelial growth inhibition was calculated by the following formula given below.

% Mycelial growth inhibition = gc-gt / gc× 100

Where,

gc = Growth of mycelial colony after incubation period in control set subtracting the diameter of inoculums disc;

gt = Growth of mycelial colony after incubation period in treatment set subtracting the diameter of inoculum disc.

RESULTS

Results suggested that antifungal activity increased with increasing concentration of elicitors and binders. Maximum activity was observed with 100% concentration. Hence this concentration was used for the preparation of herbal formulation. Among the elicitors and binders 100% neem oil cake and 100% cow dung gave 47.33 % and 31.01 % mycelial growth inhibition respectively showed in figure 1, 2 and 3.

30 ratios of herbal formulations were prepared and assayed for antifungal activity against *Alternaria solani*. According to results, Among the formulations prepared optimum activity was observed for formulation number 8, 12, 18 and 22 and percent mycelial growth inhibition is 62.92 %, 62.05 %, 92.62 %, 95.24 % respectively against *solani* showed in table 1 and figure 4. The second highest inhibition showed by formulation number 7, 10, 13, 20, 21, and 29 against *Alternaria solani*. The inhibitory activity of herbal formulation was compared with standard fungicides like mancozeb and bavistin are presented in table 2. Water used as control in which no herbal formulation and fungicides are present and all data were compared with water. On the basis of results obtained, best herbal formulation will be used for *in vivo* experiments.

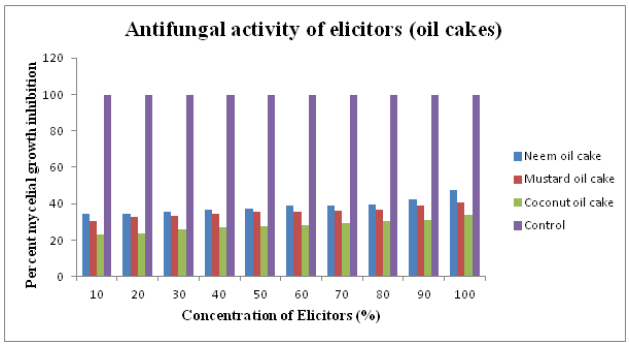

Figure 1: Antifungal Activity of Oil Cakes (Elicitors) Against Alternaria Solani

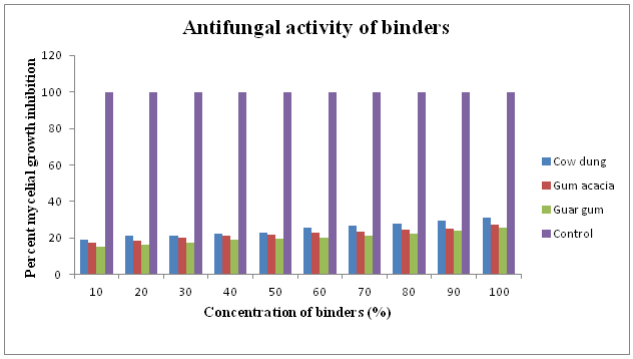

Figure 2: Antifungal Activity of Binders Against Alternaria Solani

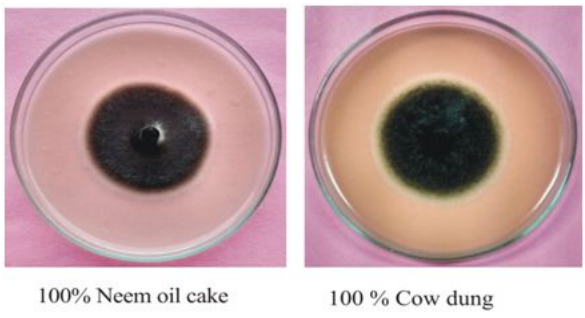

Figure 3: Antifungal Activity of Neem Oil Cake and Cow Dung

Table 1: In Vitro Antifungal Activity of Herbal Formulation Against Alternaria Solani

Ratio No	Formulation Type	Ratio	Growth Diameter after 7 days (mm) ± SD	% Mycelial Growth Inhibition
7.	100 % alcoholic crude: neem oil cake: cow dung	6:2:2	30.03 ± 0.30	60.65
8.	100 % alcoholic crude: neem oil cake: cow dung	4:3:3	28.3 ± 0.2	62.92
10.	100 % alcoholic crude: neem oil cake: cow dung	2:5:3	29.86 ± 0.47	60.88
12.	100 % alcoholic crude: neem oil cake: cow dung	2:6:2	28.96 ± 0.25	62.05
13.	100 % alcoholic crude: neem oil cake: cow dung	3:4:3	30.16 ± 0.32	60.48
18.	Chloroform extract : neem oil cake: cow dung	3:3:4	5.63 ± 0.32	92.62

Table 1: Contd.,				
20.	Chloroform extract : neem oil cake: cow dung	5:3:2	9.03 ± 0.47	88.16
21.	Chloroform extract : neem oil cake: cow dung	8:1:1	8.66 ± 0.30	88.65
22.	Chloroform extract : neem oil cake: cow dung	6:2:2	3.63 ± 0.41	95.24
29.	Chloroform extract : neem oil cake: cow dung	4:2:4	8.06 ± 0.51	89.44

Table 2: Antifungal Activity of Standard Fungicides with Water Control Against Alternaria Solani

S. No	Standard Fungicides and Water Control	Growth Diameter After 7 Days (Mm) ± Sd
1	Mancozeb	14.67 ± 1.52
2	Bavistin	35.67 ± 1.52
3	Water	76.33 ± 0.57

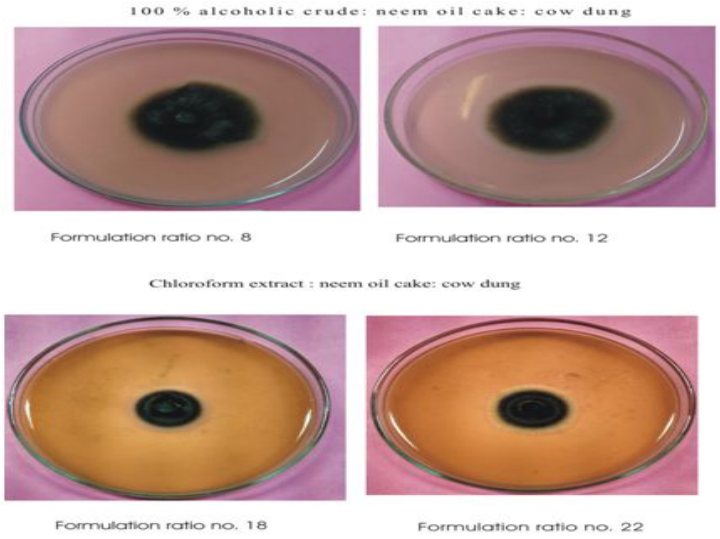

Figure 4: Antifungal Activity of Herbal Formulation

DISCUSSIONS

Plants have thousands of constituents and are important sources of new agrochemicals and biologically active molecules show antimicrobial property. Many plant and plant products have been reported as having antimicrobial activities against plant pathogenic fungi (Sokovicet, 2009). Natural products with fungicidal activity have been and are being explored in order to make fungicides which are easily biodegradable, selective and can be locally produced, especially for farmers who cannot afford expensive synthetic fungicides. At present, serious attention is drawn to extracts from higher plants that contain antifungal substances in form of secondary metabolites, which help in resisting the pathogens. The extraction of any plant material with solvent will yield a mixture of compounds. The extract may contain a wide variety of compounds like alkaloids, phenols, tannins, flavonoids, Volatile oils, Saponins and Carbohydrates etc. (Cowan, 1999).

Use and exploitation of preparations based on natural products, which can limit plant pathogens growth comes into higher and higher prominence, especially limiting synthetic fungicide/ traditional chemical application. It results from

equivalent efficiency of bio-preparations to pesticides. Plant preparations have been used for centuries in medicine and pest control. Farmers in India use neem leaves to protect their stored grain from insects. Herbs and spices, such as basil and clove, have been used by many workers to protect food from spoilage, as both have antimicrobial properties (Manohar et al., 2001).

The antimicrobial activities of *Cassia fistula* plant parts have been studied earlier by many scientists (Hajra et al., 2011; Bhalodia et al., 2012). Significant reduction in growth of pathogen like *Fusarium oxysporium, Rhizopus stolonifer* by ethanolic leaf extract of *cassia fistula* has been reported (Hajra et al., 2011). *Cassia fistula* fruit pulp extract showed antifungal activity against *Aspergillus. niger*, *Aspergillus. clavatus*, *Candida albicans* (Bhalodia et al., 2012).

Matsuzaki *et al.*, (1998) investigated that soil with cow manure amendments is ideal treatment for decreasing the severity of the disease and improving the final tubers yield of potato. Similar findings were expressed by Davis et al., 1996; Ivanyuk et al., 1996. Reduction in the stem infection has also been noticed when oats preceded potato as a green manure crop (Lootsma and Scholte, 1996). Solarizing soils plus use of appropriate organic materials have also been observed to actuate a chain reaction of chemical and microbial degradation, which increase toxicity against soil flora and fauna, especially soil borne plant pathogens. These possibly contributed to the high nutrient contents, which could be available with organic manure amendment (Gamliel et al., 2000).

Field trials against foliar and tuber disease of potato by using three types of organic material including thermal compost, static wood chips and vermin castings has been conducted by Al-Mughrabi et al. (2006). Infection of *R. solani* afflicted the normal growth and yield of potato tubers. However treatment with extract was been helpful in begin again normal yield (Khair and Haggag, 2007).

Alternaria solani is one of the most common and destructive pathogen of potato and so far no control practices have been found to manage the potato early blight disease efficiently. Though, a number of chemical compounds have been introduced in recent years to overcome this pathogen, but due to certain limitations including environmental pollution, mutagenic deterioration and ecotoxicological effects, no one could be known as ideal for effective and safe management of potato early blight. Feeling the gravity of scenario, a rapid upsurge for the development of herbal formulation has been observed in recent years to manage the harmless decline of potato crop. Several studies have pointed out the potential of neem (*A. indica*) tree to control plant pathogenic fungi that could be listed it as top fungicide and harmless bio-control agent (Abbasi et al., 2003; Dubey et al., 2009). In present study, we examined the herbal formulation which contains *Cassia fistula* fruit pulp crude and partially purified extract, neem oil cake and cow dung against *Alternaria solani* to manage potato crop.

CONCLUSIONS

Best herbal formulation will be used for *in vivo* experiments. The results of the present studies would suggest that use of herbal formulation from *Cassia fistula* L. fruit pulp extract holds promise control of early blight of potato compared to fungicides which are costly and hazardous.

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